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THE UNIVERSITY OF ALBERTA

MECHANISMS INCREASING THYROXINE REQUIREMENT OF RATS IN THE COLD

A Thesis

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF SCIENCE

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ABSTRACT

The effects of cold, and of increased intake of non-digestible food bulk on thyroid hormone requirement of the rat have been studied using I-131 labelled l-thyroxine as a tracer. The rate of thyroxine breakdown by cold exposed rats exceeded that of animals in the warm. Increasing the amount of non-digestible material consumed, either by placing rats in the cold, or by adding such material as diluent in the feed of rats in the warm, accelerated the loss of hormone in feces. The increased loss possibly occurred because of impaired absorption of thyroid hormone from the intestine. Estimates of thyroid secretion rates also reflected increased hormone losses induced by diet and cold. These findings confirmed previous suggestions that the greater amount of food eaten by rats in the cold increased thyroxine requirement by causing a more rapid fecal loss of the hormone.

Binding of thyroxine by non-absorbed particulate material in the intestine was investigated as a possible cause of increased fecal hormone loss. Cellulose flour or finely divided polyethylene were added in equal weights as non-digestible diluents to a synthetic feed of low bulk and the two mixtures were given to two groups of rats kept in a warm room at 25°C. The specific gravity of the polyethylene was lower than that of the cellulose, hence for equal weight of diluent taken in polyethylene constituted the larger volume. In contrast to cellulose the

polyethylene had little affinity for radiothyroxine and was therefore thought to influence thyroxine loss in feces only by its presence as bulk. A third group of rats were fed the synthetic diet alone. The rates of breakdown and of fecal loss of thyroid hormone by the three groups were studied using labelled thyroxine as a tracer. The rats receiving cellulose in the feed eliminated a smaller volume of feces than the animals given polyethylene, but lost thyroxine more rapidly. The rate of fecal hormone loss was considerably faster for the rats given either diluent than for the animals fed the undiluted ration. Thyroxine was found to be bound by cellulose but not by polyethylene. Feces containing polyethylene carried I-131 bound to small heavier particles, believed to be endogenous in origin, that were enmeshed in the polyethylene. Similar matter in the feces of rats given the synthetic feed alone also carried bound I-131 although of lower activity.

The phenolic hydroxyl group in the thyroxine molecule is believed to be an active binding site under physiologic conditions. Materials capable of binding thyroxine at this site may, when present in the intestine, inhibit absorption of the hormone by competition. The presence in the intestine of fecal bulk material that does not bind thyroxine may however inhibit absorption passively by hindering mixing.

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To my wife

Doreen

INTRODUCTION

The rate of thyroid hormone secretion of rats maintained in a moderately cold environment (5° to 10°C) for periods of two weeks or more is significantly greater than the secretion rate of control rats kept at room temperature. The physiological significance of this increase is not yet fully understood. The accelerated secretion may be related to development of cold acclimation since certain changes seen in acclimated rats resemble some of the changes seen in rats made hyperthyroid by administration of excess thyroid hormone. Conversely, the thyroid hyperfunction may reflect primarily a passive involvement of thyroid hormone in the elevated metabolic response of the rat to a cold environment. For example, a greater loss of thyroid hormone in the feces may be caused by increased fecal bulk as the rat exposed to cold eats more food in order to support a raised level of heat production.

The rate of secretion of hormone by the thyroid gland is controlled by thyroid stimulating hormone (TSH) released by the anterior pituitary gland. Accelerated release of TSH is known to initiate accelerated release of thyroid hormone. Elevated or diminished blood thyroxine levels inhibit or enhance TSH release respectively.

Animals in the cold secrete more TSH than controls. This may reflect stimulation of the anterior pituitary via the hypothalamic-hypophyseal pathway. Alternatively, TSH secretion in the cold reflects lowered blood thyroxine levels resulting

from accelerated breakdown and/or loss of thyroxine in the cold.

Thyroid hormone is lost more rapidly in the feces of rats maintained in the cold. The increased amount of food eaten and consequently greater bulk in the intestine has been related to the increased fecal hormone loss and has been suggested as a major cause of thyroidal hypersecretion in the cold. In addition thyroid hormone is broken down more rapidly by the cold exposed animal, and this has also been suggested as a major cause of thyroid hypersecretion.

To evaluate the significance of these two factors in bringing about the elevated thyroid hormone secretion in the cold exposed rat the rates of tissue breakdown of thyroxine and loss of the hormone in feces were studied in warm control and cold exposed rats eating normal feed and feed with increased bulk. The thyroid secretion rates of similarly treated rats were studied to complement the foregoing.

Thyroxine appearing in the feces enters the intestine in the bile. Most of that entering the intestine is absorbed and returns to the circulation. However, absorption is not complete. The amount lost may be proportional to fecal volume suggesting that bulk may impair absorption passively. In addition, binding of thyroxine by specific diet constituents might cause a disproportionate increase in hormone loss. The possibility that thyroxine binding by material appearing in the feces might influence thyroid secretion was investigated in

this study. Concentrated synthetic diets containing bulk materials known to differ in thyroxine affinity were fed to rats at room temperature and the rates of tissue breakdown of thyroxine and loss of hormone in feces were studied.

REVIEW OF RELEVANT LITERATURE

Thyroid response to cold

Thyroid secretion of small homeothermic animals is increased when they are maintained in a moderately cold environment. This conclusion has been reached by many workers and is based on a variety of evidence. Indirect indices of secretion rate including morphological, histological and cytological changes in thyroid glands have been presented as evidence for enhanced function. Increased gland weights(25), vascularization(20), follicle cell heights(35), and decreased follicular colloid(24) have been described. Each has led to the conclusion that thyroid function is augmented in animals kept in the cold.

Measurements of thyroid activity using I-131 also indicate increased secretion by rats in the cold. Leblond, Gross, Peacock and Evans(23) injected controls and rats exposed to cold for 7 to 28 days with radioiodide. They reported accumulation of I-131 in the gland (thyroid uptake) and incorporation of the isotope into thyroid hormone was more rapid in the cold exposed animals. After comparing several parameters of thyroid function in warm control rats and rats exposed to 5°C for various periods, Cottle and Carlson(6) reported that the percentage of serum protein-bound I^{131} (PBI^{131}) in the total serum I^{131} twenty-four hours after the injection of radioiodide was greater in rats cold exposed for 8 days or longer, and suggested that the difference was a consequence of increased rate of glandular

incorporation of inorganic I^{131} and increased rate of secretion of I^{131} labelled thyroid hormone. They further found that rats cold exposed for 7 days or longer released thyroidal I^{131} at a significantly faster rate than controls, and although they did not determine thyroid specific activities (S.A.), they concluded that thyroid hormone secretion rate was increased by cold exposure.

The results of studies involving measurement of thyroxine replacement dosages support the evidence for increased thyroid secretion in the cold. Chemical agents such as thiouracil block thyroid hormone synthesis and normally produce thyroid gland enlargement as a result of accelerated release of thyroid stimulating hormone (TSH) as circulating thyroxine levels fall. Dempsey and Astwood(10) administered thiouracil as a thyroid blocking agent to groups of control and cold exposed rats. They determined that the amount of exogenous thyroxine sufficient to prevent the increase of gland weight in the cold was approximately double that of warm controls and concluded that this reflected secretion rate. Woods and Carlson(44) repeated this experiment and also determined the thyroxine dosage necessary to restore resting metabolism of thyroidectomized warm and cold exposed rats to the respective intact control levels. Their findings confirmed those of Dempsey and Astwood. Similarly, Heninger, Newcomer and Thayer(16) used the "goitrogen" technique to estimate the thyroid secretion rates of chickens kept at

75°F, 95°F, and 105°F. They reported that secretion was greatest for the colder group and least for the birds kept at 105°F.

Control of thyroid secretion

Thyroid stimulating hormone

Release of thyroid stimulating hormone (TSH) is augmented in animals placed in the cold and this in turn would be expected to augment thyroid secretion. The former was shown by Bottari(4) who reported that the level of circulating TSH in the blood of cold exposed rabbits was increased shortly after the period of exposure began. Knigge(22) described the rapid acceleration of thyroidal I^{131} release that occurred in the hamster within 3 to 4 hours of placing in the cold at 5°C. This was concluded to indicate increased TSH release, as pituitary TSH stores were 60 percent depleted within 0.5 to 1 hours after placing the animals in the cold, and exogenous TSH invoked a similar response. Brown-Grant(5) reported that thyroidal I-131 release was accelerated in the rat within 8 hours of moderate cold exposure (6.5°C and 11.5°C). Stevens, D'Angelo, Paschkis, Cantarow, and Suderman(36) estimated TSH levels in serum and in anterior pituitaries of guinea pigs after various periods of exposure to cold and reported concentrations to be increased with cold exposure in both. They concluded that TSH formation and release was accelerated with cold exposure. They further found that thyroidal I-131 release rates in guinea pigs kept 6 to 8 weeks in the cold were approximately twice that of controls which supports the finding that TSH release was accelerated.

Control of TSH release

The mechanisms through which cold stimulates TSH release have not been fully defined. The initial accelerated secretion in response to acute cold exposure may occur through hypothalamic activation of the pituitary gland. Such a conclusion was reached by Uotila(41) who placed rats in which the pituitary stalks were sectioned in the cold for various periods and observed that the hyperplasia of thyroid cells seen in intact rats when placed in the cold did not occur in the operated animals. The conclusions drawn from this and similar studies by subsequent workers have been questioned because of possible hypophyseal damage. However, electrolytic lesions in the hypothalamus, particularly in the anterior and preoptic areas, have been reported to prevent the rapid thyroidal I-131 release normally seen in animals soon after exposure to cold and seem to confirm the studies in which the hypophyseal-hypothalamic stalk was sectioned. Knigge and Bierman(21) found that hamsters with acute bilateral hypothalamic lesions released thyroid I-131 at a normal rate in a warm environment, but differed from intact animals in that the rate of release did not change with 12 hours of exposure at 5°C. Rats with similar lesions showed no aberrant thyroid or pituitary function when kept at room temperature for long periods but when exposed to 5°C cold for 12 to 52 days the accelerated thyroidal I-131 release and histologic activation seen in intact controls was not

evident(9). Further suggestion that the normal hypothalamic-hypophyseal relationship is essential has been given by studies in which implants of pituitary tissue have been made in the anterior chamber of the eye of hypophysectomized animals. Von Euler and Holmgren(43) made such implants in the rabbit and reported that although thyroid I-131 release of these animals approximated control release in a warm environment, no increase in I-131 release rate took place when the graft bearing animals were exposed to cold. However, the data presented by these workers shows that at room temperature the release by the grafted animals was slower than by intact controls which questions the validity of their conclusions. However, similar conclusions were made by Knigge and Bierman(21), using the hamster. Both groups of workers concluded that the typical rodent thyroid response to acute cold was dependent upon the presence of the intact hypothalamo-hypophyseal relationship.

In addition to direct hypothalamic modulation of thyrotrophin secretion, the level of circulating thyroid hormone in the blood influences TSH secretion. Increased blood thyroxine concentrations cause inhibition of TSH release, lowered blood levels stimulate TSH release. This control mechanism is probably independent of the hypothalamic connections since Von Euler and Holmgren(43) found that administration of exogenous thyroxine inhibited thyroid I-131 release in hypophysectomized rabbits bearing intraocular

implants of pituitary gland. This negative feedback mechanism has been utilized in several techniques, such as that used by Dempsey and Astwood(10), for assessing thyroid hormone secretion. These require determination of the quantity of administered thyroid hormone necessary to fully block thyroid secretion or to maintain constant a pertinent TSH secretion rate(14,27). Increased tissue "utilization" and fecal loss of thyroid hormone by the cold exposed rat could both tend to lower blood hormone levels and thus stimulate TSH secretion. This was suggested by Rand, Riggs, and Talbot(25) who determined protein bound iodine (PBI) as a measure of circulating thyroid hormone and found that it was significantly lower in cold exposed rats. They postulated that this drop in circulating hormone stimulates TSH release.

The mechanism through which the enhanced production of thyroid hormone is achieved and maintained in the continuously cold exposed rat is not definitely known. Possibly both neural and humoral factors contribute, with initiation of the hyperactive state instituted through hypothalamic-hypophyseal stimulation of the anterior pituitary, and adequate hormone production attained and controlled through the response of the pituitary-thyroid feedback system to the stimulus of thyroxine disappearance.

Changes with acclimation to cold

Acclimation may be defined(15) as modifications induced

in an animal continuously exposed to constant temperature under laboratory conditions. Certain changes, in addition to thyroid hyperactivity, are seen in the chronically cold exposed small mammal, and appear to be related to acclimation. The markedly increased tolerance of chronically exposed rabbits and rats to severe cold has been described(3). Metabolic rates measured in the cold (5°C) and in the warm (28° to 30°C) are increased in acclimated rats as compared to non-acclimated controls(7,11). Also the calorigenic response to a given dose of adrenaline is greater in acclimated rats than in non-acclimated animals(28,37). From heat balance studies on warm and cold acclimated rats Depocas, Hart, and Heroux(11) concluded that the cold acclimated animals were able to achieve a greater production of heat and thus maintain heat balance under more severe conditions. Clearly, the significant factor pertinent to the enhanced survival of cold acclimated rats is the developed ability to maintain a high metabolic rate.

Role of the thyroid hormone

There is considerable uncertainty with respect to the degree of participation of thyroid hormone in the metabolic responses of the cold exposed animal. The rapid stimulation of thyroid secretion which one would expect through the hypothalamo-hypophyseal system suggests that the augmented secretion initiates some change in the animal. This role would seem likely since increased metabolic rates (28° to 30°C)

and increased responses to adrenaline are also seen in rats made hyperthyroid by administration of excess hormone and cold acclimated rats(28,37). However pretreatment of rats with large doses of thyroxine fails to increase cold tolerance as does acclimation(31,8).

Other findings suggest that a small proportion only of the augmented thyroid hormone secretion in the cold exposed rat may relate directly to increased metabolism and survival in the cold. Sellers and co-workers(30) placed thyroidectomized control and cold acclimated rats in the cold at 1.5°C and reported that the acclimated animals survived for an average of 34.6 days compared to 7.8 days for the controls. Further, they(29) found that thyroidectomized rats given 2.5 microgm. of thyroxine daily were able to maintain an elevated metabolic rate (1.5°C) indefinitely. This amount is equivalent only to the approximate daily secretion of rats kept at 25°C. They concluded from these findings that the development of increased metabolism by cold exposed rats was dependent on the presence of thyroid hormone and independent of the increase in thyroid gland function.

Disappearance of thyroid hormone in the cold exposed animal

Utilization and breakdown

Degradation of thyroid hormone, as evidenced by deiodination, is accelerated in rats exposed to cold. The product of deiodination has been shown to be the inorganic iodide. Kassenaar, Lameyer, and Querido(19) measured the rate of appearance of radioiodide in the urine of thyroidectomized rats given I-131 tagged l-thyroxine in lieu of the usual maintenance dosage of 5μg stable l-thyroxine daily. They reported that, during the first 24 hours after injection, the rate at which equivalent quantities of injected radiothyroxine underwent degradation was significantly faster for rats kept at 4°C than for control animals kept at 32°C. Similar results were described by Intoccia and Van Middlesworth(17) who found that rats previously exposed to 10°C for 11 days excreted an average of 40 percent of the injected radioisotope in urine within 24 hours after injection, in contrast to 32 percent excreted by animals kept at 22°C. Although breakdown of thyroxine in the tissues of the body is apparently increased in the cold exposed rat, the significance of the effect in relation to increased metabolism or cold acclimation is not known.

Flock and Bollman(12) suggested that in a warm environment much of the thyroid hormone degradation in the rat occurred in the liver. They found that excretion of urinary radioiodide within 20 hours after injection of I-131 thyroxine was markedly

reduced in eviscerated rats and inferred that the deiodinating activity of other tissues was limited. Since the liver, stomach, spleen, pancreas and intestines were extirpated in the experimental animals surgical shock may have been sufficient to obscure non-visceral thyroxine metabolism.

Circulating thyroxine may also be deiodinated in rat skeletal muscle. This is suggested by the recent isolation by Tata(38) of a material from rat muscle which deiodinates thyroxine in vitro. This material, which Tata concluded was an enzyme (thyroxine dehalogenase), deiodinated iodothyronines 20 times more readily than it did iodotyrosines, and utilized d- or l-thyroxine as substrate. Increased enzymic activity was found in muscle extracts from rats pretreated with l-thyroxine, d-thyroxine had no effect. As the animals received large hormone dosages for 4 weeks the physiological role of the enzyme is difficult to assess.

Excretion of thyroid hormone in feces

A significant proportion of the thyroid hormone produced by the rat may be lost to the organism through excretion in the feces. Albert and Keating(1) demonstrated that in the rat the liver rapidly removed injected radiothyroxine from the blood. An average of 43 percent of injected I-131 labelled l-thyroxine was found in the livers of anesthetized weanling rats within one minute after the injection. As the liver radioactivity level decreased in the following six hours an approximately

equivalent increase occurred in total stomach and intestine activity. In the normal rat most of the liver accumulated radiothyroxine entering the intestinal tract is carried in the bile(18). Chromatographic analyses have shown that thyroxine in bile is conjugated with glucuronic acid. Taurog(39) chromatographed rat bile before and after incubation with beta-glucuronidase and found that the incubated bile contained free thyroxine only. Since the butanol extract of rat feces contained free thyroxine and no conjugate he concluded that during passage through the intestine an enzymatic hydrolysis of the excreted glucuronide was effected, possibly by the beta-glucuronidase activity of intestinal bacteria. The physiological significance of the conjugation and of its breakdown is not known.

The amount of thyroid hormone actually lost in the feces of the rat over a definite interval is considerably less than the amount initially entering the intestine from the liver. Studies using labelled hormone suggest an extensive hepatic-portal and lymphatic recirculation of thyroxine absorbed from the intestine. Albert and Keating(1) injected 4.5 μg of I-131 thyroxine into the small intestine of rats with patent and ligated bile ducts. After 48 hours the collected feces of the ligated animals contained approximately 33 percent of the injected dose, hence two-thirds of the radiothyroxine was considered to have been absorbed. During the same interval

the normal rats excreted approximately two-thirds of the injected radiohormone, which suggested that although both groups probably absorbed comparable amounts, in the normal rats at least one-half of this had been recirculated to the liver and excreted again.

Triantaphyllidis(40) considered fecal excretion to be the primary avenue of thyroid disposal in the rat. In a study of the proportion of an injected amount of radiothyroxine that was excreted in the feces of man, the rabbit and the rat, she suggested that the percentage of fecal thyroxine varied with mean fecal volume per Kg. body weight. This was greater in the small species which produce a large fecal volume for their size. Thus, within a species the fecal loss of thyroid hormone would relate closely to mean fecal volume. Semi-starved rats produced a smaller volume of feces and released thyroidal I-131 more slowly than ad lib fed controls(26), and rats fed a high bulk diet excreted injected radiothyroxine in the feces more rapidly than controls given a lower level of dietary bulk(2).

Cold exposed rats have been shown to excrete administered I-131 labelled thyroxine in the feces more rapidly than warm controls. This was reported by Kassenaar et al(19) who found that the cold exposed rats excreted 16.0 percent of the injected radiothyroxine within 24 hours as compared to 6.1 percent excreted by the rats kept at 32°C. They suggested that the absorption of thyroxine was impaired in the cold exposed

animals. Further, Intoccia and Van Middlesworth(17) reported that fecal loss of administered I-131 thyroxine was enhanced in cold exposed rats. Relating this to the greater amount of food eaten by the animals in the cold they suggested that the increase in fecal thyroxine loss caused by greater fecal bulk in the cold would contribute significantly to thyroid hypersecretion in the cold.

Statement of problem

The effects of cold, and of food bulk intake and fecal volume, on thyroid hormone metabolism were investigated in the present study. Rats kept in the cold and in the warm were given either a "normal bulk" cereal feed, or a "high bulk" feed which consisted of the normal cereal diet diluted 20% wt./wt. with alphacel (Nutritional Biochemicals Co. - brand of non-nutritional cellulose flour). The animals given the high bulk diet ate more of this feed apparently to achieve adequate caloric intake(32,33). Consequently the daily volume of the feces of these animals was considerably increased compared to the rats at the same temperature eating the cereal diet alone. The increase in fecal volume of the rats in the warm fed the diet diluted with alphacel, and the cold exposed rats given the normal cereal diet, was comparable with respect to the fecal volume of the control animals in the warm eating the cereal feed alone.

The rates of tissue breakdown of thyroxine and loss of the hormone in feces of 4 groups of rats kept under the above

experimental conditions were studied using I-131 labelled l-thyroxine as a tracer. The distribution of labelled hormone in endogenous thyroxine was considered equivalent in all animals for 24 hours after injection so that during this period the rates at which the groups lost I-131 in feces and in urine allowed comparison of the rates of excretion and breakdown of endogenous hormone. Further, as the animals were considered to be in a steady state with respect to rates of thyroid hormone excretion and secretion, the I-131 losses in 24 hours for each group were considered proportional to the respective rates of secretion of thyroid hormone.

The effects of cold and fecal bulk on thyroid secretion rate were evaluated in other groups of warm and cold exposed rats fed the experimental diets as above. The measurement of thyroid secretion rates was attempted using the method developed for individual rats by Grosvenor and Turner(14). Quantitative measurement using this method was found during this study to be subject to considerable deviation which could not be evaluated because the data and included error were interrelated by their technique. However, after recalculation to obtain discrete data, allowing statistical treatment, the effects of cold and fecal bulk upon thyroid secretion rates were evaluated.

The influence of alphacel upon fecal loss of radioactivity appeared to be greater than would be attributable to bulk alone. As radiothyroxine in water mixtures of alphacel, feces containing

alphacel, and cereal feed, was found to be taken out of solution, the possibility that thyroid hormone was lost by binding to certain fecal materials was investigated. Rats in the warm were fed a synthetic diet of very low bulk. Other rats in the warm received this diet diluted 30% wt./wt. with alphacel, or with finely divided polyethylene. As the polyethylene did not retain radiothyroxine in water mixture, and was also considerably greater in volume per equal weight than alphacel, the influence of this material on fecal thyroid hormone loss was considered to be as bulk only. Also studied were the rates of tissue breakdown and loss of thyroid hormone in the feces of rats given these 3 diets using I-131 labelled l-thyroxine as a tracer.

METHODS

General

Male Sprague-Dawley rats ranging from 350 to 450 grams in weight were used. All were housed individually in metal cages. Equivalent periods of illumination and darkness were automatically provided. Food and water were given ad lib. When food intake was measured, the food can was weighed together with the food before it was placed in the cage and again after 24 hours, the difference represented the food consumed. It was then filled again to the previous weight. Spilling was found to be minimal if only a slight excess of food was given. For collection of urine and feces the animals were placed in circular metabolism cages mounted over 8" polyethylene funnels that drained into 100 ml. Erhlenmeyer flasks. Feces were collected on screens of 1/6" mesh suspended into the funnel body. Before collections were made two days were allowed for the animals to adjust to the cages. All feces collected were dried 24 hours and weighed. Except when on a feeding regime the rats were fed a homogeneously ground cereal feed*. The composition of this feed was as follows:

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* A modification of Miracle Dog Chow, Ogilvie Flour Mills Ltd., Edmonton, Alberta.

Table 1. Cereal Feed (Ogilvie Mills Special Mix)

Ingredients	% by wt.
Ground Wheat	24.0
Wheat Germ	11.0
Soya Bean Meal	11.0
Meat Meal	15.0
Whey Powder	2.5
Fish Meal	2.0
Dried Brewer's Yeast	2.0
Cereal Grass	2.0
Vitamin E Wheat Germ	5.0 oz./ton
Multi-Vitamin Mix	5.0 lb./ton
Vit.A	1.25×10^6 units/lb.
Vit.D_3	0.5×10^6 units/lb.
Riboflavin	750 mg./lb.
Ca. Pantothenate	200 mg./lb.
Vit.B_{12}	1 mg./lb.
Niacin	5 gm./lb.
Iodine Analysis*	5 ppm.

* A. L. Chaney Laboratory Inc.

Thyroid hormone excretion, secretion, with equal bulk in warm and cold

Preliminary experiments using alphacel as a non-nutritional food diluent had established that rats could maintain normal growth, as measured by body weight gain, when given the cereal feed diluted up to 30% wt./wt. with alphacel. In addition, rats kept at 25°C given the cereal feed diluted 20% wt./wt. with alphacel produced daily fecal volumes closely comparable (as dry weights) to those of rats kept at 5°C receiving the cereal feed undiluted.

Two groups of six rats were kept at 5°C for 21 days prior to the study and two groups were kept as warm controls at 25°C.

One group at each temperature received the cereal feed undiluted, the other groups were fed the cereal feed diluted 20% wt./wt. with alphacel. Seven days were allowed for the animals to adjust to the diets, then food intakes were recorded daily for a further seven days. The body weights were initially measured twice weekly, and then were measured every two days from the day prior to the experiment.

Each rat was injected subcutaneously with 0.2 ml. of isotonic* saline containing 0.25 μg of I-131 labelled l-thyroxine (S.A. - approximately 30 μc/μg). Urine and feces were collected at 24 hour intervals for 96 hours after the injection. At each collection the flasks were changed, and the screens and funnels washed.

All radioactivity was counted as gamma emission using a scintillation well counter (Nuclear Chicago) and decade scaler (Tracerlab). Duplicate counts were taken, each of one minute duration, corrected for background and averaged for expression as net counts per minute (net c/m). All samples counted were at constant volume of 10 ml., and were contained in 15 ml. graduated glass centrifuge tubes. A 10 ml. standard containing 1% of the injected dose was counted at the same time as the excretion samples. The ratio of $\frac{\text{sample net c/m}}{\text{standard net c/m}}$ multiplied by an aliquot factor (A.F.) was used to calculate the sample activity as a percentage of the injected dose. The use of the standard corrected for decay of the isotope and

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*containing 0.1% Na_2CO_3

minimized variation due to changes in counting efficiency.

To determine the radioactivity lost in the urine the volume collected per day for each animal was made up to 50 ml. with water and a 10 ml. aliquot was taken for counting. To test if organically bound I-131 was present (thyroxine) in the urine, one mg. of bovine plasma protein was mixed with a second 10 ml. aliquot. After 5 minutes the protein was precipitated with 20% trichloroacetic acid (TCA). The supernatant was removed after centrifuging for ten minutes and the precipitate was alternately washed with 10% TCA and centrifuged three times. The precipitate was then dissolved in sodium hydroxide, made up to 10 ml. and counted as a measure of organic I-131. The supernatant and washings were pooled and 10 ml. aliquots were counted to determine inorganic I-131. The percentage of injected radioactivity excreted in the urine in 24 hours was calculated as follows:

$$\begin{array}{l}\text{Total activity}\\ \quad\text{\% dose}\end{array} = \frac{\text{urine aliquot net c/m}}{\text{standard net c/m}} \times \text{A.F. (urine)}$$

$$\begin{array}{l}\text{Organic activity}\\ \quad\text{\% dose}\end{array} = \frac{\text{urine aliquot "ppte" net c/m}}{\text{standard net c/m}} \times \text{A.F. (urine)}$$

$$\begin{array}{l}\text{Inorganic activity}\\ \quad\text{\% dose}\end{array} = \frac{\text{urine aliquot supernatant net c/m}}{\text{standard net c/m}} \times \text{A.F. (urine)} \times \text{A.F. (supernatant)}$$

To determine the fecal loss of radioactivity the feces of each animal collected each day after weighing were suspended

in 75 or 100 ml. of water using a Waring blendor. An aliquot of approximately 8 ml. was taken. This was centrifuged to determine more exactly the total volume, the volume of the solids, and the volume of the liquid portion in order to calculate the A.F. (feces). To test if inorganic I-131 (iodide) was present in the feces, the aliquot was then mixed thoroughly with 1 ml. of solution containing 1 mg. of bovine plasma protein and after 5 minutes precipitated with 20% TCA. The precipitated residue was centrifuged and washed as above, then made up to 10 ml. with sodium hydroxide and counted as a measure of labelled hormone. The supernatant and washings were pooled and a 10 ml. aliquot counted to measure inorganic I-131.

Calculations of the percentage of injected radioactivity excreted in a 24 hour feces collection required that the A.F. (feces) be ascertained. The A.F. (feces) as the ratio of $\frac{\text{volume of feces + water}}{\text{volume of aliquot}}$ was derived from the following relation:

$$\frac{\text{aliquot water volume}}{\text{aliquot total volume}} = \frac{\text{water volume added to feces}}{\text{total volume of feces + water}}$$

Therefore A.F. (feces) =

$$\frac{\text{total volume of feces + water}}{\text{aliquot total volume}} = \frac{\text{water volume added to feces}}{\text{aliquot water volume}}$$

As before, the percentage of injected radioactivity excreted in 24 hours was calculated:

$$\frac{\text{Total I-131}}{\text{\% dose}} = \frac{\text{fecal aliquot net c/m}}{\text{standard net c/m}} \times \text{A.F. (feces)}$$

$$\text{Organic I-131 \% dose} = \frac{\text{fecal aliquot residue net c/m}}{\text{standard net c/m}} \times \text{A.F.(feces)}$$

$$\text{Inorganic I-131 \% dose} = \frac{\text{fecal supernatant aliquot net c/m}}{\text{standard net c/m}} \times \text{A.F.(feces)} \times \text{A.F.(supernatant)}.$$

An evaluation of thyroid secretion rate was attempted by the method of Reineke and Singh(27) as developed for single animals by Grosvenor and Turner(14). This entailed in vivo counting of I-131 activity over the region of the thyroid gland. In this experiment anesthetic was not used as this was believed to be hazardous for the cold exposed animals.

Four groups of six rats each were maintained as described above with respect to diets and temperature conditions except that in this study the two cold exposed groups were kept at 5°C for 28 days.

Each animal was injected intraperitoneally with approximately 20 μc of I-131 as NaI. After 96 hours the thyroidal radioactivity was measured. Each animal was held firmly by hand, prone on a 1/4" lead shield. Positioning of the thyroid region over the scintillation well counter crystal was accomplished by alignment with a plexiglass covered aperture using ear and nose guide marks on the shield. The error involved in positioning was randomized, and the possibility of operator bias reduced, by removing and repositioning the animals after each count. Initially the

animals were held for one minute, removed, and repositioned for a second one minute count. Two successive counts within the "standard probable percent counting error" (68%) of the highest count were accepted. When the rat struggled the count was not recorded and the procedure was repeated. Experience showed that the rats usually did not struggle during the first 30 seconds. For this reason the counting period was shortened to 1/2 minute. Repeated counts were made until three showed agreement. The thyroid region activity was corrected for background and expressed as mean counts per minute. Counts were made every 48 hours. Commencing immediately after the initial count the animals were injected subcutaneously daily with a measured amount of stable l-thyroxine contained in 0.2 ml. isotonic* saline. The thyroxine dosage was increased after each counting, as shown below:

Table 2 Thyroxine dosage - counting

Day:	1	2	3	4	5	6	7	8	9
Count:	Initial		2		3		4		5
Dosage thyroxine $\frac{\mu g}{100 \text{ gm.bwt.}}$	1.0	1.0	1.5	1.5	2.5	2.5	3.0	3.0	

The method is based on the assumption that the degree of inhibition of thyroidal I-131 release by a given dose of thyroxine is directly related to the amount of endogenous thyroid hormone normally secreted. Therefore, if a series of graded

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*containing 0.1% $Na_2 CO_3$

doses of stable thyroxine are given and the degree of inhibition is plotted, the dose required for 100% inhibition can be estimated by extrapolation. To estimate 100% inhibition the mean thyroid radioactivity determined at a particular measurement for each animal is expressed as a percentage of the preceding count and is plotted against the thyroxine dosage received over the corresponding interval. From the resulting points joined by regression to form a straight line the amount of thyroxine required to completely inhibit I-131 release is obtained graphically. This quantity is considered to be equivalent to the endogenous hormonal secretion rate.

This means of analysis proved unsatisfactory. During the analysis of data obtained, doubt arose regarding the validity of the expression of thyroid radioactivity as a percentage of the previous count since this made it possible that a relatively small error in a count might invalidate this count and the subsequent one. The points plotted for individual rats (4 counts - 3 points) deviated sufficiently from linearity that a straight line could not validly be fitted. For this reason each activity measured was recalculated as a percent of initial count and was then expressed as the difference, in percent, from the initial count. After transformation from percent to arc sine angles by the conversion of Snedecor(34), the data for the first three thyroxine levels were examined by means of a "simple factorial" analysis of variance. The

sources of variation considered, and the degrees of freedom are listed below.

Table 3 Analysis of Variance, factors and degrees of freedom

Sources of Variation	Degrees of Freedom
Temperature	1
Diets	1
Thyroxine level	2
Temp. x Diet interaction	1
Temp. x Thyroxine level interaction	2
Diet x Thyroxine level interaction	2
Temp. x Diet x Thyroxine level triple interaction	2
Error (plus replicates)	60
TOTAL	71

The analysis tested the null hypothesis that alterations in rate of disappearance of thyroid radioactivity effected by temperature, diet, or thyroxine dosage, or by interaction of these factors, were not significantly different than those attributable to random variation.

Thyroxine binding by non-digestible material

In testing whether or not thyroxine was bound to materials used as diluents, radiothyroxine was added to various materials, or was initially present as in feces carrying excreted I-131. After homogenization in water each mixture was repeatedly washed with water at pH 7. The degree of binding was considered to be inversely related to the ease with which the radioactivity could be removed by washing. The degree of thyroxine binding thus evaluated was compared for different materials subjected to the same number of washings. In each instance, after homogenization, duplicate aliquots were taken of each labelled material. The radioactivity initially present was measured, then the aliquots were centrifuged and the supernatant decanted, water was again added to form a second mixture and was removed by centrifuging. The washing process was continued through 4 to 10 sequences depending on the nature of the material. The radioactivity of the washed residues, resuspended to original volumes, was determined and expressed as a percentage of the activity originally present.

I-131 thyroxine was added to water mixtures of alphacel, cereal feed, and vermiculite (exploded mica - insulating material). The radioactivity was counted before and after 5 washings.

Radiothyroxine was added to water mixtures of alphacel, vermiculite, and finely ground polyethylene. The radioactivity

was counted before and after 5 and 8 washings. Three drops of household detergent were added to the polyethylene as a wetting agent.

I-131 thyroxine was added to water mixtures of feces of rats eating the synthetic diet (see below) in pure form, the synthetic diet diluted 30% wt./wt. with alphacel, and the synthetic diet diluted 30% wt./wt. with polyethylene. The mixtures were allowed to stand for 24 hours, and were then centrifuged and washed 6 times. The radioactivity was counted before and after washing. The fecal material containing polyethylene was further separated into heavy and floating fractions, the activity of these fractions was also determined.

The fecal material of rats that had been injected with radiothyroxine was also examined to determine if it bound thyroxine. The feces of rats fed the synthetic diet diluted 30% wt./wt. with alphacel, and the synthetic diet diluted 30% wt./wt. with polyethylene were examined. The radioactivity was counted before and after 10 washings. Heavier particles, freed from the fecal material containing polyethylene by centrifuging, were drawn from the bottom of the centrifuge tubes with a syringe fitted to a long hypodermic needle.

To determine if binding of thyroxine to fecal materials as well as fecal bulk could lead to increased fecal loss of hormone, rats were fed diets diluted with finely divided polyethylene or alphacel in equal weights. The animals given

the polyethylene dilution diet ingested considerably greater bulk, weight for weight the volume of polyethylene was 1.62 times the volume of alphacel. As the cereal diet contained bulk of unknown nature, a synthetic diet of little or no indigestible bulk was used instead as a basic ration to which indigestible bulk was added. The composition of this ration, given in table 4 below, was adapted from that developed by Sibbald, Bowland, Robblee, and Berg(33).

Table 4. Synthetic diet.

Ingredients per Kg. of mixed feed

Casein	203 gm.
Sucrose	688 gm.
Corn oil	50 gm.
Mineral mix*	50 gm.
Vitamin mix*	9 gm.

Three groups of six rats were kept at room temperature (26 ± 2°C). One group was fed the undiluted synthetic diet, a second group received the synthetic diet diluted 30% wt./wt. with alphacel, and the remaining group was fed the synthetic diet diluted 30% wt./wt. with finely divided polyethylene. After seven days for adjustment to the new diet, the food intake of each animal was measured daily for six days prior to the experimental period and during the four day duration of the experiment. The animals were weighed every two days for

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* Composition of Vitamin and Mineral mixes - Appendix

twelve days to ensure that normal growth was maintained on the diets, they were also weighed initially and at termination of the four day experimental period.

The animals were injected subcutaneously with 0.25 μg of l-thyroxine labelled with I-131 (S.A. 31.1 μc/μg). The excreta was collected over 24 hour intervals until 96 hours after injection.

To determine the I-131 loss in the urine during each interval, for each animal the urine collected was made up to 50 ml. with water, a 10 ml. aliquot was taken and counted as previously for total I-131 excreted. Each 24 hour urinary I-131 was expressed as percentage of injected dose.

For determination of fecal I-131 excretion the feces collected from each animal each day were oven dried for 18 to 20 hours at 60°C and weighed. The feces containing alphacel or polyethylene were powdered while dry using a Waring blendor. 1.5 gm. aliquots of the powdered feces were made up to a 10 ml. suspension using a suitable solvent, and counted for total I-131 excreted. The I-131 in feces excreted by each animal in 24 hours was expressed as a percentage of the injected dose.

The feces of animals given the synthetic diet alone were hydrolyzed in concentrated sodium hydroxide. A small amount of alphacel was added so as to form a uniform 10 ml. suspension and to simulate the counting conditions obtained for the

other fecal samples. The fecal samples were counted for total I-131 excreted. The activity excreted in feces by each animal per 24 hour period was expressed as a percentage of the injected dose.

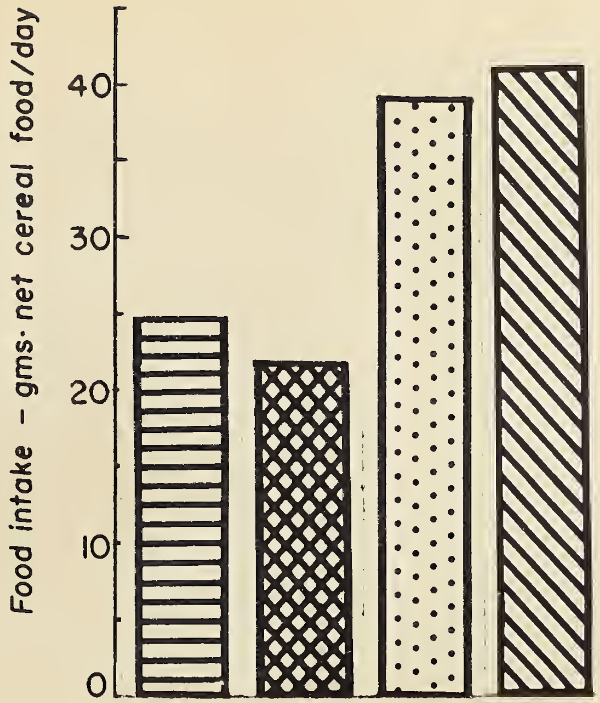

Figure I

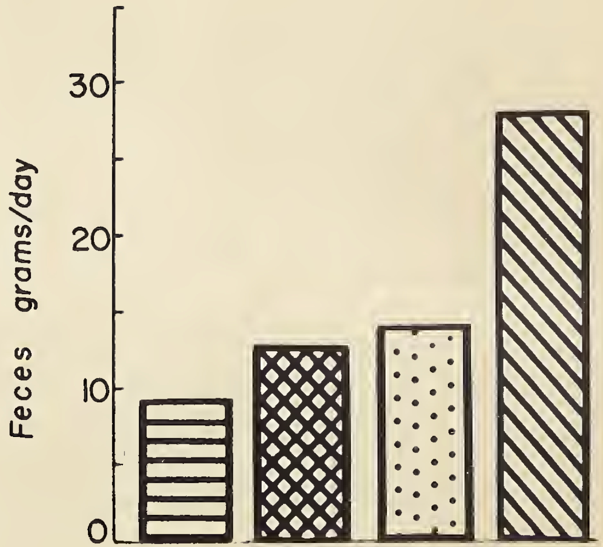

Figure 2

Average food intake (I) and fecal weight (2) of rats fed two diets in warm and cold

▤ warm cereal diet
░ cold " "
▦ warm alphacel diet
▨ cold " "

RESULTS

Thyroxine excretion, secretion, with equal bulk in warm and cold

Cold exposed rats and rats fed the cereal diet diluted with alphacel excreted injected radioactivity, as urinary and fecal I-131 combined, more rapidly than warm control animals. Rats kept at the same temperature ate approximately the same amounts of the cereal feed (fig. 1) with the result that the animals given the diet containing alphacel ate more of the diluted feed. As seen in figure 2 the "warm" rats given the diluted feed produced fecal weights comparable to those of the cold exposed group receiving the cereal feed undiluted. The constancy of the amount of the cereal feed eaten (net) by the groups in the cold and in the warm can be seen in table 5. During the 24 hours after injection of I-131 thyroxine the animals exposed to cold excreted more of the radioactivity in the urine than either of the warm groups, and also lost more I-131 in the feces than the warm rats fed the cereal diet without added diluent (table 5). The fecal I-131 loss of the warm rats fed the alphacel diluted feed was comparable with that of the cold group given the cereal feed alone.

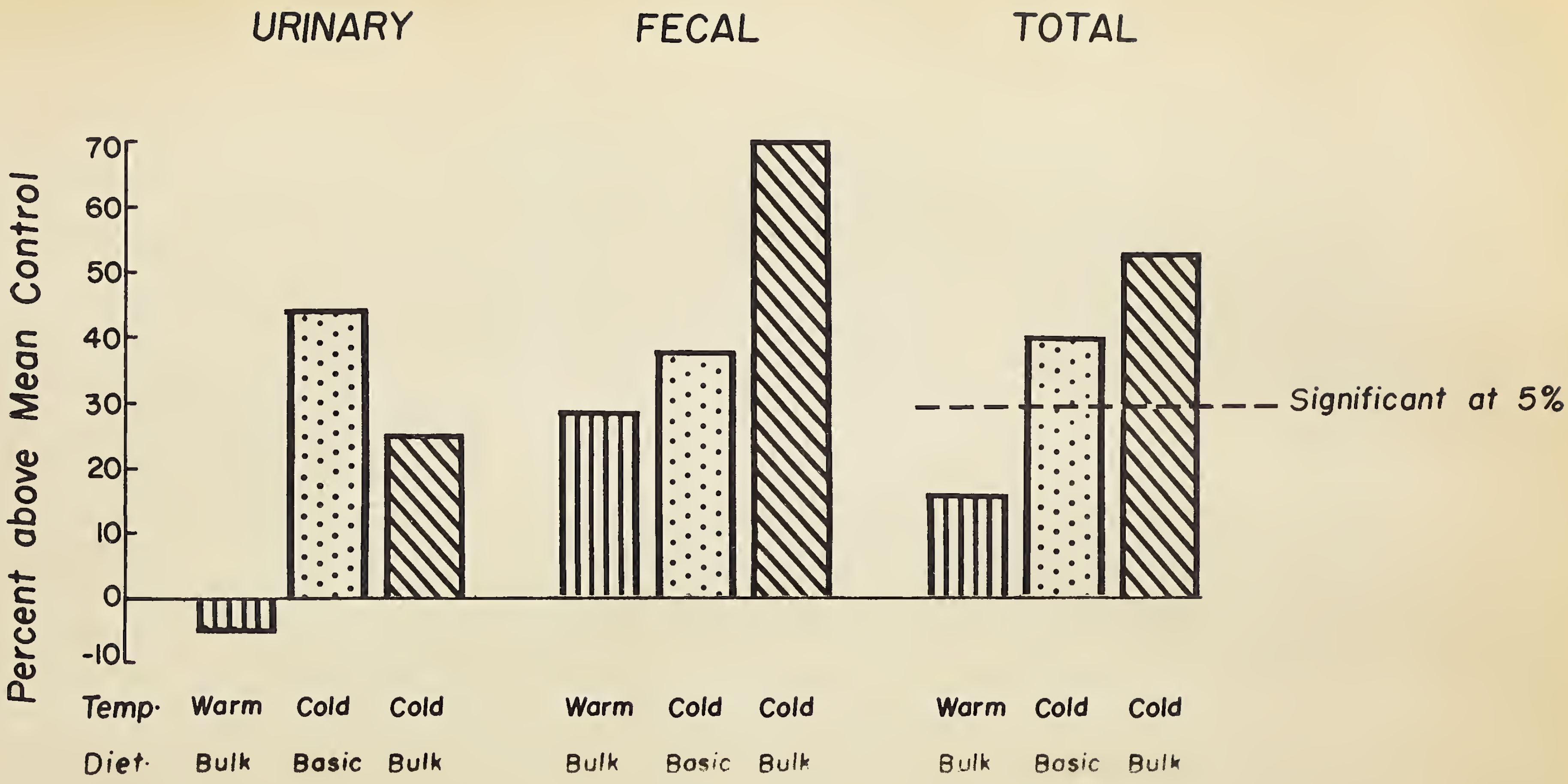

Figure 3. Urine, fecal, total I^{131} excreted 0-24 hrs., as percentage greater or less than controls in the warm.

Table 5. Mean[⊖] food intake, fecal weight, I-131 excretion after I-131 thyroxine injection of rats in warm and cold fed two diets.

Period	Group	Food	Diluent	Feces	I-131 Recovery		
		Basic			Urine	Feces	Total
hrs.		gm.	gm.	gm.	% dose	% dose	% dose
0-24	W.N.*	25.3		10.2	14.4	23.2	37.6
24-48		23.1		9.0	10.6	26.0	36.6
48-72		24.7		6.9	4.5	11.0	15.5
72-96		23.6		8.0	2.2	4.2	6.4
0-24	W.A.	22.6	5.7	14.0	13.7	30.0	43.7
24-48		22.5	5.6	13.7	9.8	33.3	43.1
48-72		21.2	5.3	11.1	3.9	12.2	16.1
72-96		22.2	5.5	12.1	1.6	4.2	5.8
0-24	C.N.	39.5		14.1	20.7	32.1	52.8
24-48		39.8		13.3	8.3	21.4	29.7
48-72		35.6		11.9	2.1	5.3	7.4
72-96		41.3		15.7	0.6	1.5	2.1
0-24	C.A.	38.9	9.7	29.9	18.0	39.5	57.5
24-48		41.8	10.5	25.7	7.4	28.0	35.4
48-72		38.8	9.7	24.7	1.7	6.2	7.9
72-96		43.4	10.9	30.4	0.4	2.3	2.7

* Code: W-warm, C-cold, N-normal diet, A-alphacel diet

[⊖] Mean ± S.D. - see Appendix

The rate of combined urine and fecal I-131 loss (total I-131) by the warm rats fed the alphacel dilution diet exceeded by 16% the rate of total I-131 loss of the warm control rats given the cereal feed alone (figure 3). Similarly, the rates of total I-131 excretion of the cold exposed rats fed the cereal diet and the feed diluted with alphacel were greater than the

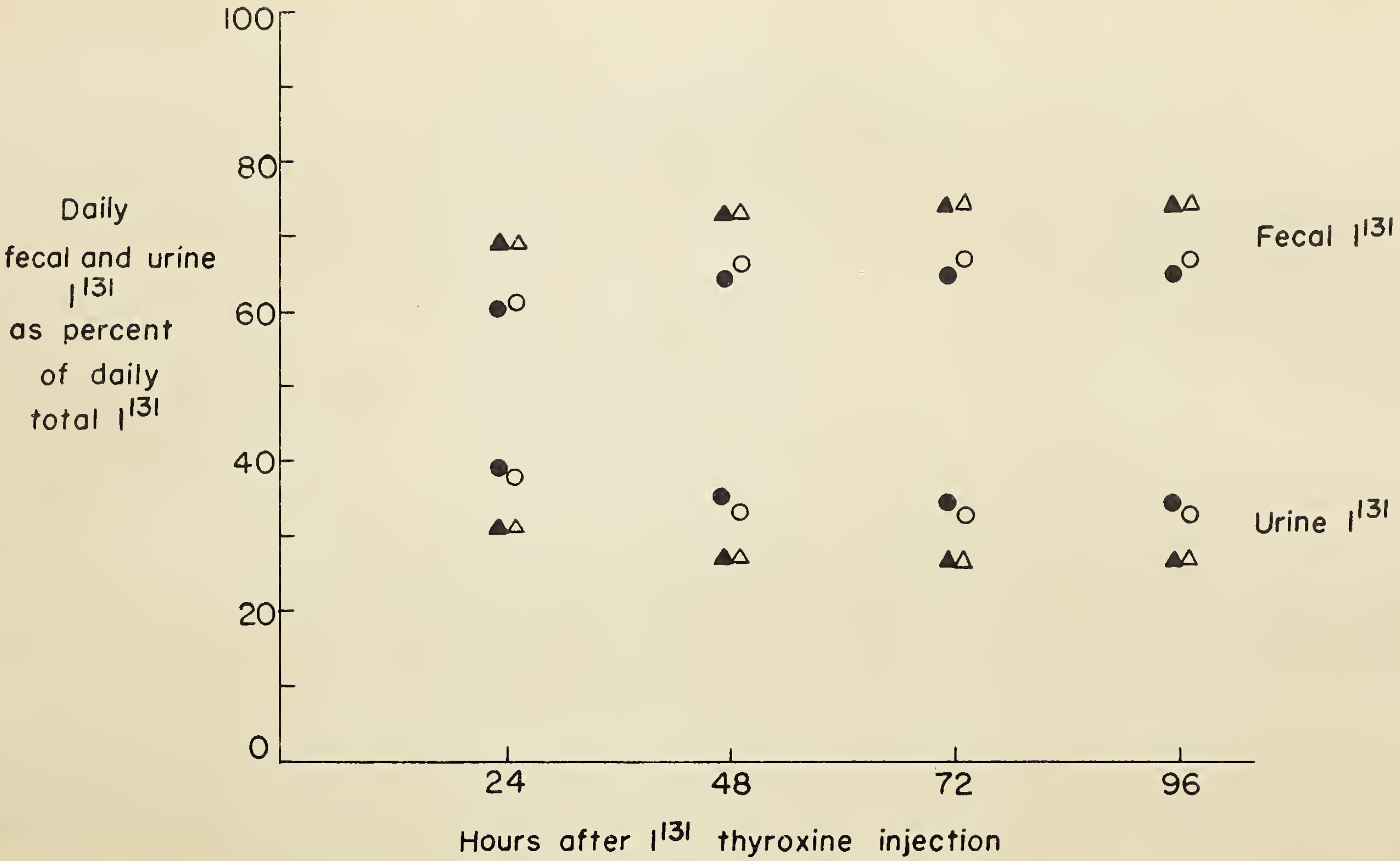

Figure 4. Urine and fecal I^{131} as percent of total I^{131} excreted each day.

○-warm cereal diet, △ - warm alphacel diet
●-cold " " , ▲ - cold " "

control rate by 40% and 53% respectively.

Alphacel in the diet enhanced fecal I-131 excretion, the urinary I-131 excreted by the animals fed the diet containing alphacel was in all instances slightly less than that excreted by the animals at the same temperature that were given the undiluted cereal feed (table 5, figure 3). Further, as seen in table 6 and figure 4, the rats receiving alphacel in the feed lost in feces the same proportion of the total I-131 excreted daily.

Table 6. Mean urine and fecal I-131 excretion.

Group	Urinary I-131 as % of day Total 0-24	24-48	48-72	72-96	Fecal I-131 as % of day Total 0-24	24-48	48-72	72-96
W N	38.3	33.8	33.1	33.0	61.7	66.2	66.9	67.0
W A	31.3	27.2	26.6	26.7	68.7	72.8	73.4	73.3
C N	39.2	35.2	34.6	34.4	60.8	64.8	65.4	65.6
C A	31.3	27.3	26.9	26.6	68.7	72.7	73.1	73.4

Code: W-warm, C-cold, N-normal diet, A-alphacel diet

In contrast, the animals eating the cereal diet alone lost a smaller and variable proportion of total I-131 in feces. This reflected also the greater urinary I-131 excretion in the cold.

Determination of the thyroid secretion rates of warm and cold exposed rats fed two levels of diet bulk was attempted using the method developed for individual animals by Grosvenor and Turner(14). However, the expression of thyroid radioactivity

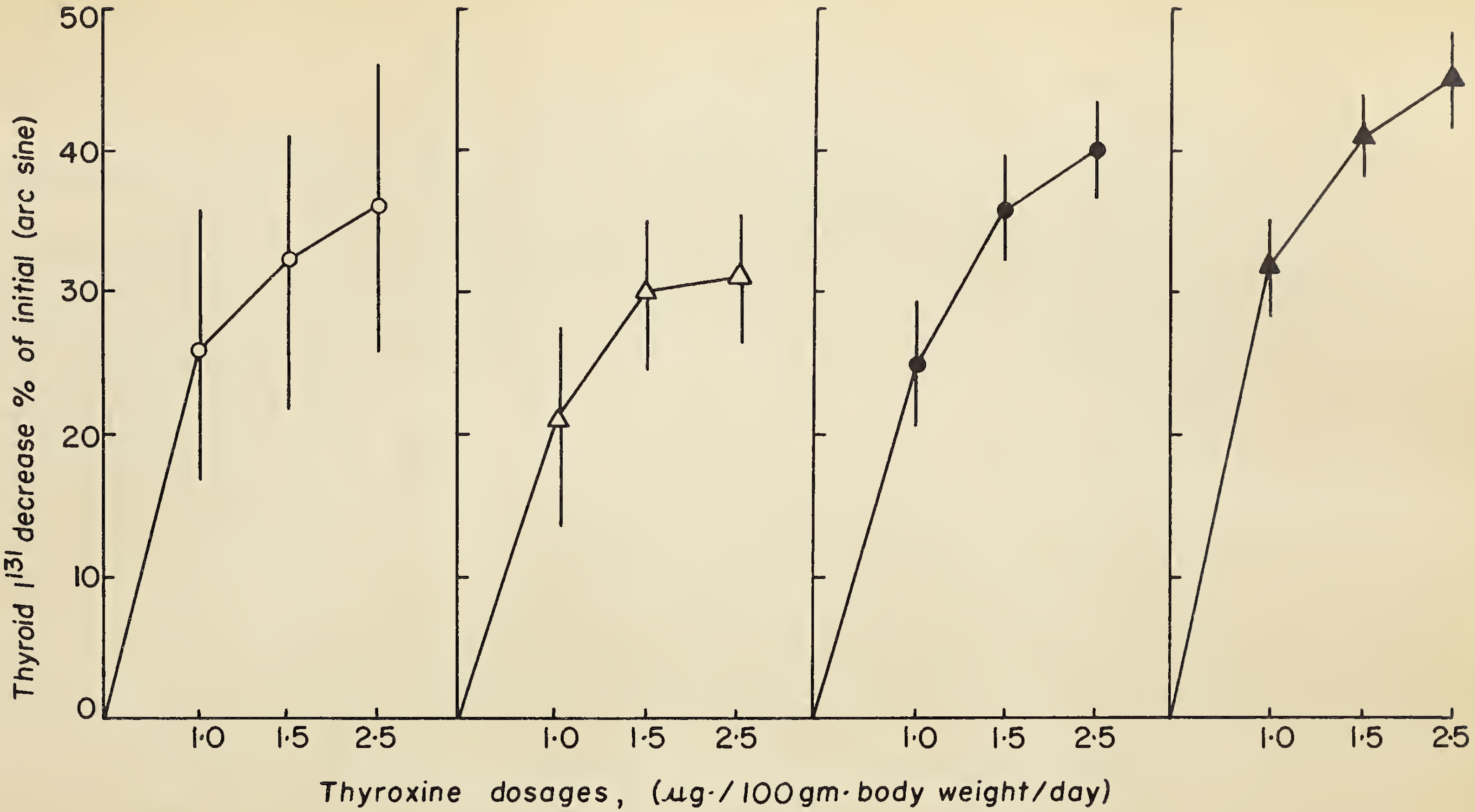

Figure 5· Mean percent decrease from initial thyroid I^{131} after three thyroxine dosages, rats fed two diets in warm and cold·

Vertical lines: ± S·D·

○ - warm, cereal diet
● - cold, " "
△ - warm, alphacel diet
▲ - cold, " "

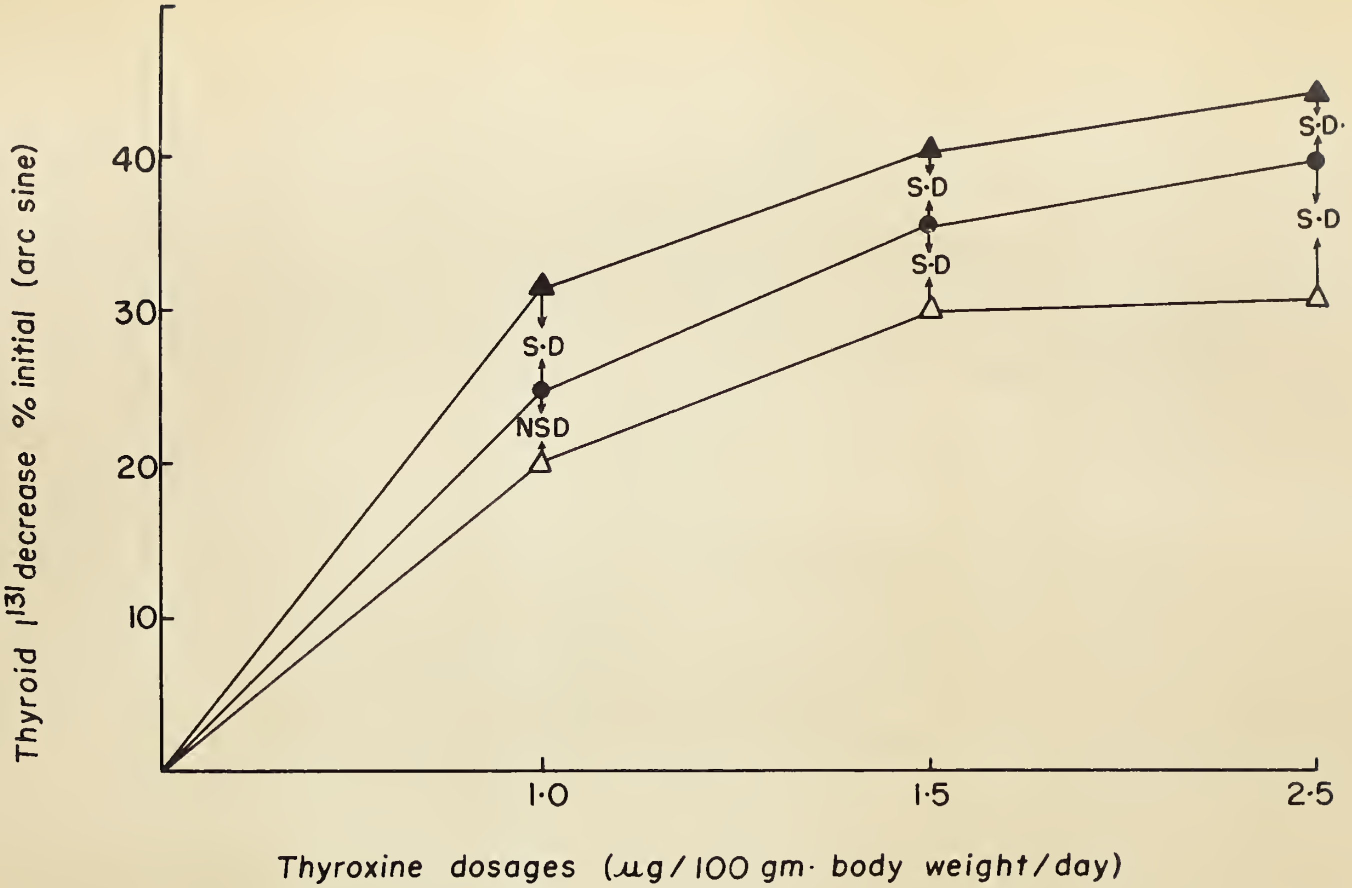

Figure 6· Mean percent decreases from initial thyroid I^{131} at three thyroxine dosages, of rats in warm and cold fed two diets·

S·D — Significant difference between means ($p < 0{\cdot}05$)

N·S·D·– No " " " " ($p \geqq 0{\cdot}05$)

△ - warm, alphacel diet

▲ - cold, " "

● - cold, cereal diet

measurements as percentages of previous counts interrelated the data and made it possible for considerable deviations to arise from relatively small counting errors. Therefore the thyroid region radioactivity measurements, expressed as percentage differences from initial counts, at the different thyroxine dosage levels were evaluated by analysis of variance (see Appendix - IV) with respect to the influence of environmental temperature and diet bulk. The analysis indicated that temperature and diet factors alone did not significantly influence thyroid secretion, but that the effect of the two factors interacting was significant. The lack of significant effect by temperature or diet bulk was unexpected, therefore the group means for three thyroxine levels and overall were compared using Fisher's(13) "t" test (see Appendix - V). There was marked variability of response in the warm control diet group at each thyroxine dosage (figure 5), thus significant differences between the means of the controls and the other groups were not seen. However, the differences between the means of the other three groups were significant (P 0.05) as seen in figure 6, with the exception of the comparison at the lowest thyroxine dosage between the warm rats fed the alphacel dilution diet and the rats in the cold given the cereal feed alone.

Thyroxine binding by non-digestible materials

Radiothyroxine in water mixtures of various diluent materials and feces was bound in some manner by certain of these and was thus taken out of solution. As seen in table 7 alphacel or feces containing alphacel retained a large fraction of the radioactivity originally present despite a number of washings with water. The feces of rats fed the synthetic feed also retained much of the radiothyroxine added. In contrast, added I-131 thyroxine was relatively easily removed from polyethylene. The radioactivity retained by fecal material containing polyethylene was not held by the polyethylene but rather by other substances.

Table 7. Retention of I-131 thyroxine.

Radiothyroxine added to:	Mean percent of initial activity remaining			
	Number of washings			
	5	6	8	10
Cereal feed	78			
Alphacel	82			
Vermiculite	54			
Alphacel	100		87	
Vermiculite	50		39	
Polyethylene	5			
Feces- synthetic diet		79		
Feces- synthetic plus alphacel diet		74		
Feces- synthetic plus* polyethylene diet		55		
I-131 excreted with:				
Feces- synthetic plus alphacel				50
Feces- synthetic plus polyethylene**				24

* These washed residues were separated into "heavy" and "floating" fractions. 49% of the initial activity was found in the "heavy" fraction and approximately 6% in the "floating" fraction.

** The normal fecal matter was not readily removed from the polyethylene mass. Repeated centrifuging and removal of the heavier material lowered the activity of the polyethylene. Little radioactivity was removed with the washings.

The rats fed the diet diluted with polyethylene consumed the same mean weight of diluent per day as the animals given the feed diluted with alphacel (table 8). Because of the lower specific gravity of polyethylene the animals receiving this material in the feed eliminated a considerably larger fecal volume each day than the rats given the alphacel dilution diet, however the latter group each day lost more I-131 in feces. The fecal radioactivity loss of the rats eating the synthetic diet alone was less than that of the other diet groups, but was highest when expressed per unit weight of feces produced (figure 7).

Table 8. Mean food intake, fecal weight, I-131 excretion (% dose) of rats fed three diets.

Group	Food		Diluent	Feces	% I-131 recovered at 96 hours		
	gross gm.	net gm.	gm.	gm.	Feces % dose	Urine % dose	Total % dose
N*	14.5	14.5		0.4	29.1	34.5	63.6
P	24.2	17.0	7.2	8.2	41.1	22.9	64.0
A	23.8	16.7	7.2	7.3	49.5	23.7	73.2

* code: N-undiluted synthetic diet, P-polyethylene diluted diet, A-alphacel diluted diet.

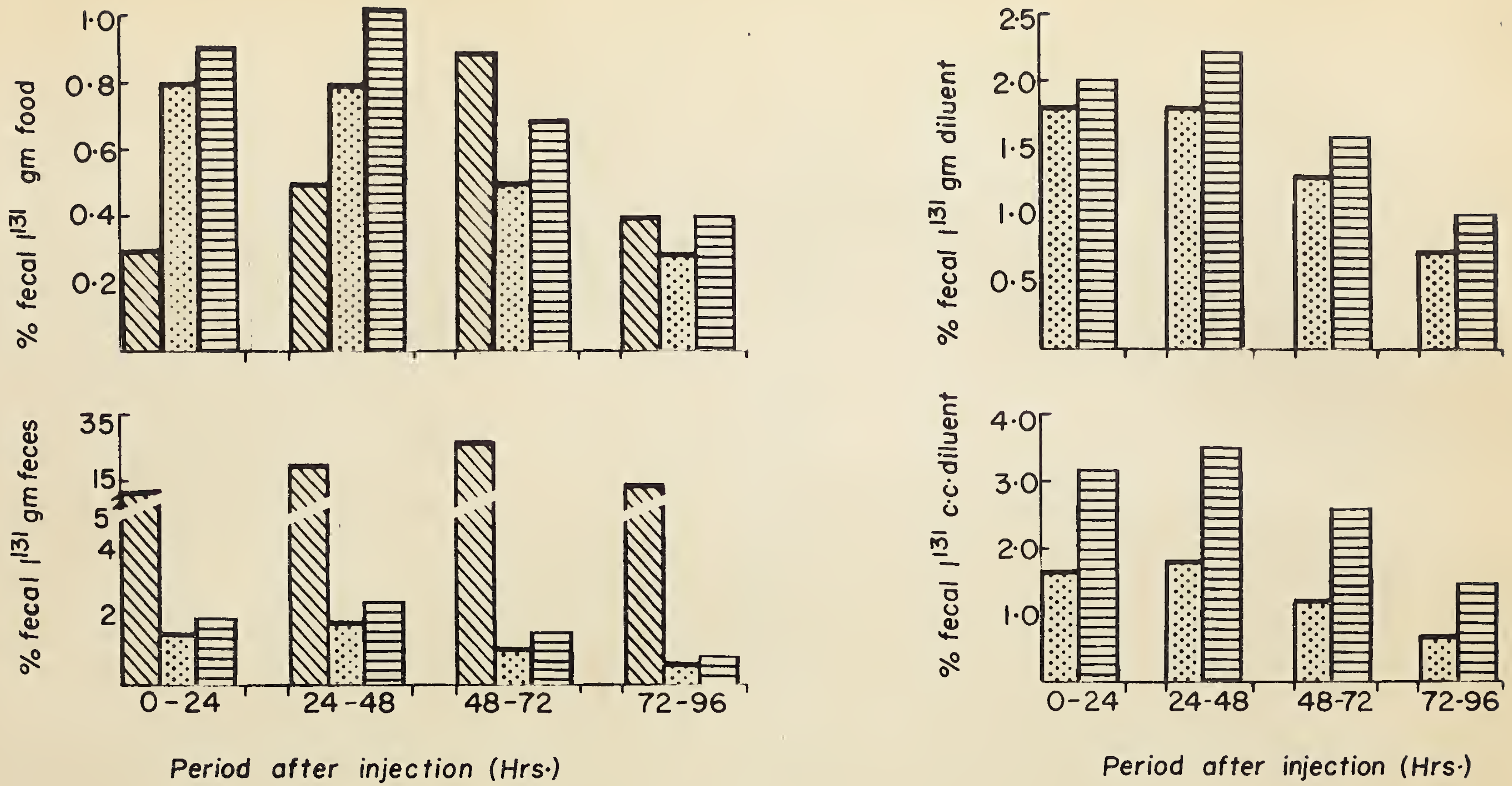

Figure 7· Mean fecal I^{131} per gm·synth·ration, diluent, feces, c·c·diluent, after rats fed three diets were injected with I^{131} thyroxine·

▧ - synthetic diet ░ synthetic diet + polyethylene ☰ synthetic diet + alphacel

Table 9. Mean* food intake and I-131 excretion each day of rats fed three diets, following I-131 thyroxine injection.

Collection Interval	Group Diet	Food gms. net	Diluent gms.	Diluent cc.	Feces gms	Fecal I-131 % of dose	Urine I-131 % of dose	Total I-131 % of dose
0-24 hrs.	N	13.7			0.5	3.8	15.0	18.8
	P	16.7	7.3	7.3	8.1	12.7	8.1	20.8
	A	18.3	7.8	4.9	8.3	15.6	9.4	25.0
24-48 hrs.	N	15.6			0.4	8.4	9.6	19.0
	P	18.3	7.8	8.0	8.1	14.2	7.5	21.7
	A	17.5	7.5	4.7	6.9	16.6	7.5	24.1
48-72 hrs.	N	13.1			0.4	11.1	5.8	16.9
	P	16.5	7.1	7.3	8.4	9.0	4.5	13.5
	A	15.8	6.8	4.3	7.3	11.0	4.4	15.4
72-96 hrs.	N	14.5			0.4	5.8	4.2	10.0
	P	16.4	7.0	7.2	8.3	5.2	2.7	7.9
	A	15.2	6.5	4.1	6.7	6.2	2.5	8.7

* code: N-undiluted synthetic diet, P-polyethylene diluted diet, A-alphacel diluted diet.

* Mean ± S.D. - see Appendix - VI.

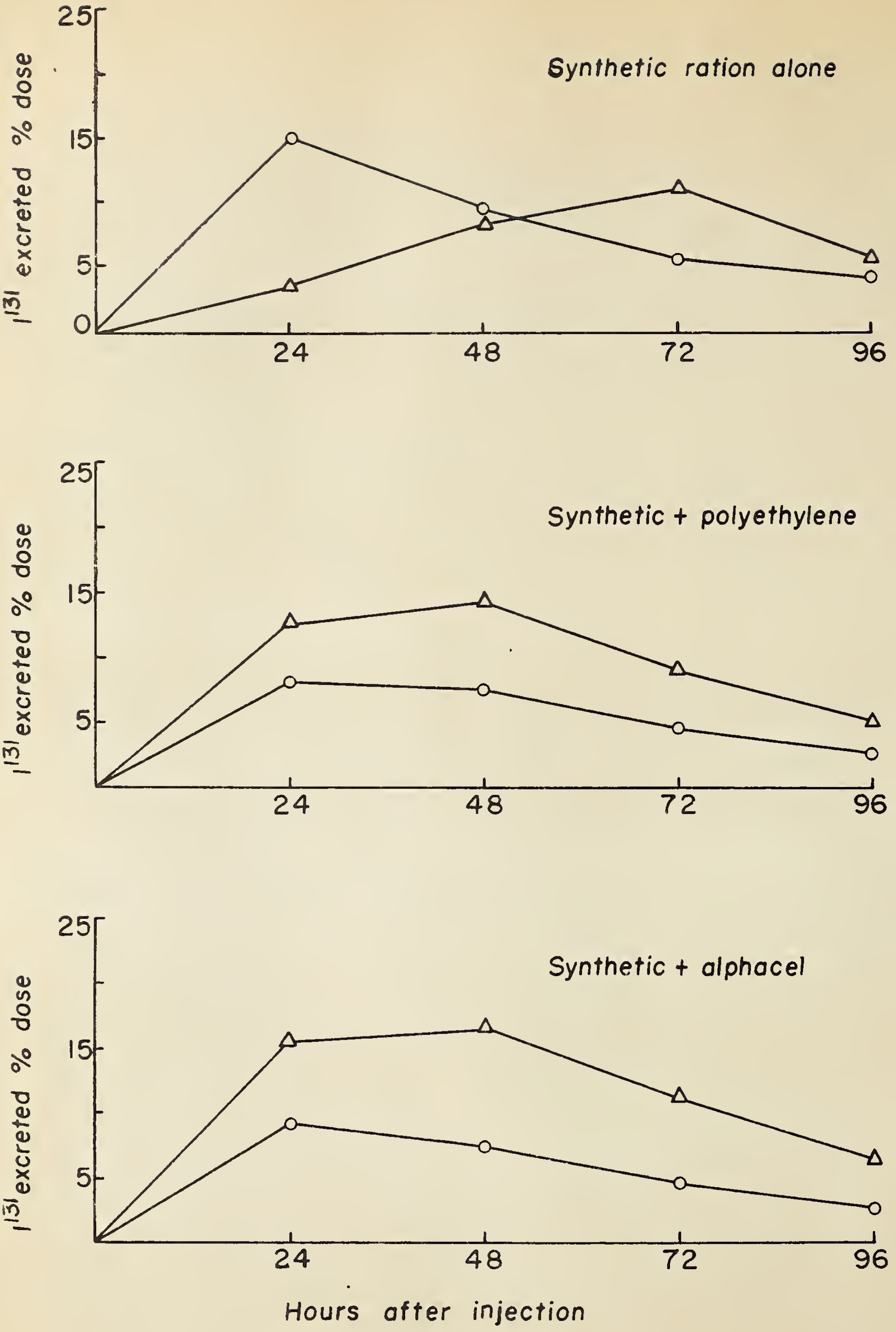

Figure 8· Mean I^{131} excretion (% dose) in urine and feces each day following injection of I^{131} thyroxine by rats fed three diets

○ - Urine I^{131}
△ - Fecal I^{131}

In spite of the greater volume of feces produced by rats fed the diet diluted with polyethylene as compared to that of the animals given the alphacel dilution diet, there was less radioactivity each day in the feces of the polyethylene fed rats expressed as the total, or per gram of food or diluent consumed, per gram of feces, or per cc. of diluent ingested (table 9, figure 7).

The rats fed the synthetic diet alone excreted urinary and fecal I-131 in a pattern differing from that seen for the animals eating either of the diluted feeds (figure 8). The fecal I-131 losses of the rats given the undiluted feed increased more slowly and were less in total than for the bulk fed rats. In contrast, the urine I-131 losses of the former group increased more rapidly and were greater in total than the urine I-131 losses of the alphacel or polyethylene diet groups.

DISCUSSION

The use of I-131 labelled thyroxine as a tracer assumes that the endogenous thyroxine pool is similar in all groups compared. Although thyroid secretion rates of cold exposed rats are increased PBI estimates of animals in the steady state in the cold and in the warm indicate little or no difference in levels of hormone circulating in the blood(6,36). Hence, assuming that the blood volumes of the various groups compared did not differ greatly, the thyroxine pools can be considered to have been similar.

During the 24 hour period following tracer injection the rates of appearance of the labelled hormone in blood from the site of injection, and of entry into the intestine in bile were assumed not to differ between groups. Differences in rates of hormone loss in the feces thus would reflect differences in the amounts absorbed from the intestinal contents. For example, as may be seen in figure 8, during the 24 hours after injection fecal I-131 must have been extensively absorbed from the intestinal tract of the rats fed the synthetic diet alone. Further, much of the I-131 absorbed may have been retained in the entero-hepatic circulation and secreted again in the bile, as evidenced by the greater fecal I-131 loss of these animals during the second and third days after injection. However, some absorbed hormone may in addition have re-entered the systemic circulation and been deiodinated since the I-131

excreted in the urine of the rats fed the synthetic diet exceeded that found in urine of the rats fed the other diets. These differences in the amounts of thyroid hormone absorbed and recirculated or lost in the feces by the various groups limit the time after injection of tracer thyroxine during which meaningful comparisons between groups can be derived from I-131 excretion rates. Maximum validity can be obtained for comparison of excretion rates by considering only the 24 hour period after injection.

Previous suggestions that the greater amount of food eaten by rats in the cold causes increased fecal losses of thyroid hormone have been confirmed. Increasing the fecal bulk, either by placing rats in the cold or by adding non-digestible material to the feed of rats in the warm, accelerated the rate of I-131 loss in feces, probably by impairing absorption of thyroid hormone from the intestine.

Although fecal loss of thyroid hormone has previously been suggested to be proportional to fecal volume(40), because increased non-digestible material mass hampered absorption of thyroxine from the intestine, binding of thyroxine to certain nonabsorbed particulate material in the intestine could cause greater loss in feces than expected from bulk alone. The results of the studies shown in table 7 suggest that radiothyroxine may be bound by cellulose. The more rapid excretion of labelled hormone in the feces of rats fed the synthetic diet

diluted with alphacel probably reflects this form of binding. However, the I-131 in feces containing polyethylene did not appear bound to this material, but rather to small heavier particles enmeshed in the polyethylene. These particles superficially resembled material of the feces of the animals given the synthetic diet alone. In both groups this material contained most of the I-131 activity present. It was presumably endogenous in origin since all constituents of the synthetic diet should have been absorbed from the intestine. The finding that more I-131 was bound to this material when polyethylene was present suggests that normally some thyroid hormone is removed from this binding material and absorbed, and that this removal and subsequent absorption is hindered by the presence of polyethylene bulk.

The phenolic hydroxyl group of the thyroxine molecule is largely ionized even at neutral pH., and according to Edsall and Wyman(11a) would be available as an active binding site. Binding of this group by sites on the material in the intestine probably occurs in competition with the absorbing process. Possibly the presence of passive bulk in the intestine would reduce thyroxine contact with the intestinal wall, and so tend to maintain bonds between non-digestible materials and thyroxine. The rate of fecal thyroid hormone loss by rats is thus a product of the non-digestible bulk consumed and of the binding properties of the

bulk, and reflects environmental temperature only through variation in food consumption.

In contrast, the accelerated rate of breakdown of thyroxine in cold exposed rats was induced only by cold and was independent of diet. The small differences in the amounts of urine I-131 excreted by rats kept at the same temperature on different diets probably reflected differences in the I-131 thyroxine available rather than rate of breakdown.

The relationship of the accelerated breakdown of thyroxine to the role of the hormone in the response of the rat to cold is not certain. More rapid breakdown may reflect physiological action of the hormone which suggests that the increased metabolism of the rat in the cold is mediated by thyroid hormone. On the other hand, the increased breakdown may merely reflect the accelerated turnover of various body materials that occurs with increased metabolic activity. This second alternative suggests that the role of thyroxine is permissive. Such a conclusion was reached by Sellers and You(29) upon findings that acclimation to cold could develop in thyroidectomized rats given small maintenance dosages of thyroxine, and that subsequent to acclimation such rats could survive without additional hormone for a considerable period in the cold. Similarly treated non-acclimated rats succumbed rapidly in the cold.

Although the significance of the accelerated breakdown of thyroxine in the cold exposed rat may be in doubt, from the

present studies loss of hormone by this means may represent a considerable portion of thyroxine requirement of rats kept in the cold.

Thyroid secretion, as reflected by thyroid I-131 release in figure 6 was greatest in the group of rats kept under the same conditions as the animals that degraded and excreted I-131 labelled thyroxine most rapidly.

The findings presented support the suggestion that increased thyroxine requirement, and consequent secretion, of rats in the cold occurs because of accelerated hormonal breakdown in the tissues of cold exposed rats, and as a product of increased fecal hormone loss as a result of greater consumption of food including more non-digestible bulk. The binding activity of the non-digestible bulk constitutes a further variable with respect to fecal hormone loss of the cold exposed rat.

CONCLUSIONS

The thyroxine requirement of rats kept in the cold is believed to be increased because of accelerated hormonal breakdown and increased loss of the hormone in feces.

The significance of the increased breakdown of thyroxine that occurs in the cold exposed rat is not known.

Increased loss of thyroid hormone in feces of cold exposed rats is believed to be a result of greater food consumption and consequent increased intake of non-digestible material by these animals.

Thyroxine in the intestine of the rat is considered to be bound by ingested cellulose thereby hindering absorption and causing greater loss of the hormone in feces.

In addition to losses caused by binding of thyroxine, the physical mass of non-digestible materials present in the intestine is believed passively to hamper absorption of thyroxine.

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APPENDICES

Table I - Synthetic Diet Composition

Ingredients per Kg. mixed Feed

Casein	203 gm.
Sucrose	688 gm.
Corn oil	50 gm.
Mineral mix	50 gm.
Vitamin mix	9 gm.

Mineral Mix: grams %

$CaCO_3$	29.29
$CaHPO_4\cdot 2H_2O$	0.43
KH_2PO_4	34.31
NaCl	25.06
$MgSO_4\cdot 7H_2O$	9.98
$Fe(C_6H_5O_7).6H_2O$	0.623
$CaSO_4.5H_2O$	0.163
$MnSO_4\cdot H_2O$	0.121
$ZnCl_2$	0.02
KI	0.0005
$NH_4)_6Mo_7)_{24}\cdot 4H_2O$	0.0025
	100.0000 gm.

Vitamin mix: gm./Kg. mixed Feed

Water soluble mix	2.5 gm.
Fat soluble mix	5.0 gm.
Choline chloride	1.5 gm.
	9.0 gm.

Water Soluble Vitamin mix/Kg. Feed

Thiamine HCl	5.0 mg.
Riboflavin	5.0 mg.
Nicotinic Acid	25.0 mg.
Ca Pantothenate D	20.0 mg.
Pyridoxine HCl	2.0 mg.
Biotin - cryst.	0.1 mg.
Vit. B_{12} (+I.F.)	0.02 mg.
Vit. K (menadione)	0.5 mg.
Sucrose	2.441.88 mg.
	2.5 gm.

Fat Soluble Vitamin mix/Kg. Feed

Tocopherol. DL.	100 mg.
Vit. A 400 I.U.	20 mg.
Vit. D 200 I.U.	5 mg.
Corn Oil	4875 mg.
	5.0 gm.

Table II. Mean food intake, fecal weight, I-131 excretion after I-131 thyroxine injection of rats in warm and cold fed two diets, with variation shown.

Collection	Group T D	Food		Feces		Urine I-131		Fecal I-131		Total I-131
		Net	± S.D.	gms.	± S.D.	% dose	± S.D.	% dose	± S.D.	% dose
0-24	W.N.	25.3	4.9	10.2	2.3	14.4	2.9	23.2	3.6	37.6
24-48		23.1	3.4	9.0	1.3	10.6	1.4	26.0	2.7	36.6
48-72		24.7	4.0	6.9	1.4	4.5	0.6	11.0	2.5	15.5
72-96		23.6	3.5	8.0	1.6	2.2	0.4	4.2	1.1	6.4
0-24	W.A.	22.6	2.1	14.0	2.0	13.7	1.6	30.0	2.8	43.7
24-48		22.5	2.2	13.7	2.2	9.8	1.2	33.3	7.1	43.1
48-72		21.2	2.9	11.1	1.7	3.9	0.6	12.2	2.1	16.1
72-96		22.2	2.3	12.1	1.9	1.6	0.1	4.2	0.9	5.8
0-24	C.N.	39.5	4.1	14.1	1.5	20.7	1.2	32.1	6.0	52.8
24-48		39.8	3.7	13.3	1.6	8.3	1.3	21.4	1.4	29.7
48-72		35.6	4.1	11.9	1.5	2.1	0.4	5.3	1.7	7.4
72-96		41.3	3.5	15.7	2.3	0.6	0.1	1.5	0.5	2.1
0-24	C.A.	38.9	3.4	29.9	5.9	18.0	2.4	39.5	7.0	57.5
24-48		41.8	4.4	25.7	2.5	7.4	0.9	28.0	7.9	35.4
48-72		38.8	5.8	24.7	4.7	1.7	0.3	6.2	1.6	7.9
72-96		43.4	6.2	30.4	3.2	0.4	0.1	2.3	1.3	2.7

Code: W-warm, C-cold, N-normal diet, A-alphacel diet

Table III - Thyroid I-131 decrease of rats fed two diets in warm and cold, given three thyroxine dosages, with variation shown.

Temp.Diet	% Difference	Number of Rats/group						Mean ± S.D.	
		1	2	3	4	5	6		
W N	d_1	26.42	32.20	38.23	29.33	18.44	12.25	26.14	9.4
	d_2	31.76	37.94	41.73	37.47	24.04	20.79	32.29	8.5
	d_3	35.91	43.22	45.69	43.05	27.06	19.91	35.81	10.3
W A	d_1	19.09	26.21	30.33	9.63	17.05	20.44	20.46	7.2
	d_2	33.21	35.37	35.30	24.58	25.62	25.40	29.91	5.2
	d_3	31.56	36.15	36.51	24.88	27.28	27.97	30.72	4.8
C N	d_1	24.50	29.47	24.88	19.19	20.96	30.79	24.96	4.6
	d_2	30.20	38.94	37.70	33.46	35.00	39.47	35.80	3.6
	d_3	36.15	44.43	40.46	38.12	36.93	43.45	39.92	3.4
C A	d_1	33.46	32.30	30.92	25.55	36.15	32.08	31.73	3.5
	d_2	38.82	39.06	41.96	41.32	44.20	38.23	40.60	2.3
	d_3	43.57	43.74	44.03	44.31	49.49	40.92	44.34	2.8

code: W-warm, C-cold, N-normal cereal diet, A-alphacel diluted diet

d - percent difference (as arc sines) from initial thyroid I-131 counted in vivo.

d_1 - percent difference after 1.0 µg thyroxine/100 gm. body wt./day for 2 days

d_2 - percent difference after 1.5 µg thyroxine/100 gm. body wt./day for 2 days

d_3 - percent difference after 2.5 µg thyroxine/100 gm. body wt./day for 2 days

Table IV. Analysis of Variance - Thyroid Secretion Rates.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	"F"	"F" Value Required for Sig.	
					5%(*)	1%(**)
Temp. (T)	1	882.63	882.63	2.08	161.0	4052.0
Diets (D)	1	4.04	4.04	0.01	161.0	4052.0
Diff. (d)	2	1825.79	912.90	24.91**	3.15	4.98
T x D Interaction	1	424.23	424.23	11.58**	4.00	7.08
T x d Interaction	2	43.95	21.97	0.60	19.47	99.48
D x d Interaction	2	7.20	3.60	0.10	19.47	99.48
T x D x d Interaction	2	20.91	10.46	0.28	19.47	99.48
Error (+ reps.)	60	2198.77	36.65			
TOTAL	71	5407.51				

Variance analyzed to determine if factors of temperature (T), diet bulk (D), or interacting combinations of these factors exerted significant effects upon the rate of change of thyroidal radioactivity (d) produced by the administered dosages of stable thyroxine.

Significance (1%) of (d) factor was inherent in the design.
Significance (1%) of T x D interaction:

T x D - Mean Square used as Valid Error to determine "F" values of (D) and (T) factors.

Table V. "t" Test - Thyroid Secretion Rates.

Experimental Groups Compared		Treatment Effect compared	Dosage of Stable thyroxine (μg/100 gm. body wt.) received 48 hours prior to I-131 assay			
Temp.	Diet		1.0	1.5	2.5	Total Response
Warm Warm	normal alphacel	Diet	NSD*	NSD*	NSD*	NSD*
Cold Cold	normal alphacel	Diet	SD	SD	SD	SD
Warm Cold	normal normal	Temperature	NSD*	NSD*	NSD*	NSD*
Warm Cold	alphacel alphacel	Temperature	SD	SD	SD	SD
Warm Cold	normal alphacel	Interaction	NSD*	SD	NSD*	SD
Warm Cold	alphacel normal	Interaction	NSD	SD	SD	SD

NSD - No significant difference between means. p 0.05

SD - Significant difference between means. p 0.05

* - Excessive variance contributed by warm, normal group.

Table VI. Mean food intake and I-131 excretion each day of rats fed three diets, following I-131 thyroxine injection, showing variation.

Collection interval	Group	Food		Feces		Fecal I-131		Urine I-131		Total I-131
	Diet	Net gm.	± S.D.	gm.	± S.D.	% of dose	± S.D.	% of dose	± S.D.	% of dose
0-24 hrs.	N	13.7	2.5	0.5	0.3	3.8	2.0	15.0	1.9	18.8
	P	16.7	2.1	8.1	1.1	12.7	2.4	8.1	1.0	20.8
	A	18.3	1.6	8.3	0.9	15.6	2.4	9.4	1.2	25.0
24-48	N	15.6	0.9	0.4	0.1	8.4	2.8	9.6	1.2	19.0
	P	18.3	2.5	8.1	1.4	14.2	1.7	7.5	1.5	21.7
	A	17.5	2.1	6.9	0.7	16.6	1.4	7.5	0.6	24.1
48-72	N	13.1	1.6	0.4	0.06	11.1	5.1	5.8	1.1	16.9
	P	16.5	1.4	8.4	0.8	9.0	1.5	4.5	0.7	13.5
	A	15.8	1.9	7.3	0.7	11.0	1.5	4.4	0.2	15.4
72-96	N	14.5	0.9	0.4	0.09	5.8	1.5	4.2	1.1	10.0
	P	16.4	1.5	8.3	0.8	5.2	1.0	2.7	0.4	7.9
	A	15.2	2.5	6.7	1.3	6.2	0.8	2.5	0.2	8.7

code: N-undiluted synthetic diet, P-polyethylene diluted diet
A-alphacel diluted diet.

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